because of the inconstancy in amount of salt in a salt-marsh during the growing season.—M. A. CHRYSLER, *The University of Chicago.*

CHROMOSOME REDUCTION IN LILIUM CANADENSE.

THE investigations, a brief résumé of whose results is given below, were carried on upon the dividing pollen mother-cells of *Lilium canadense* L., collected in the vicinity of Madison during the past five summers. A fuller account, with figures, is practically completed, but as it must be some time before it appears in print, and as the results are in some points quite different from those obtained by any previous observer, it seems advisable at this time to publish a brief statement of my observations.

After the completion of the division which forms the pollen mother-cell, there is a long period during which the cell and nucleus increase greatly in size. The nucleus contains during this period, in addition to the nucleoles, numerous irregular masses of considerable size, connected by narrow strands or fibers, the whole forming an extremely irregular network. In preparations stained with Flemming's triple stain, the larger masses show an affinity for the safranin, the fibers for the violet. There are also numerous short, fine, blue-staining fibers attached to the larger bodies, giving the network a ragged appearance.

This general arrangement persists until just before the passage of the nucleus into the condition of synapsis, when the blue-staining fibers begin to grow longer and to become more uniform in thickness; at the same time the larger masses or knots decrease in size. While these changes are going on, it is evident in many portions of the network that two fibers lie side by side and parallel; sometimes such parallel strands are attached at their corresponding ends to the same red-staining mass. While this rearrangement or pulling out of the nuclear material into threads is going on, all of the chromatic nuclear substances become massed against one side of the nuclear membrane, resulting in the synaptic figure so often described. The formation of the spirem is not fully completed until after the occurrence of this eccentric massing; there is, therefore, in this case no "dolichonema" stage preceding synapsis.

As has been said, while the spirem is being formed, it is seen in many places to consist of two parallel threads; and this continues to be the case until all of the staining substance within the nucleus, excepting the nucleoles, has been distributed along the spirem. In

other words, the spirem is double; and its double nature results, not from an early longitudinal split, but from the presence of two separate and distinct threads.

The two threads are in general parallel; in many places they are loosely twisted about each other, in others they diverge more or less widely. Almost immediately after their complete formation they approach each other more closely, become appressed and twisted about each other, and finally fuse into a single thread whose appearance gives no evidence of its double origin.

After the formation of the two threads and before their fusion, each one shows in favorable material (especially if stained with Heidenhain's iron-alum-haematoxylin) a differentiation, noted by many writers, into darkly-staining chromatin bodies (chromomeres) and a more lightly-staining ground substance, or linin. As the threads approach each other, they become so arranged that in general, though with occasiona exceptions, each chromomere in one thread lies opposite one in the other thread; and the fusion of the threads is followed by a fusion of the chromomeres in pairs. The single thread formed by the fusion then contains a row of chromomeres which show no sign of their double origin; but each original chromomere, as well as each one resulting from the fusion, is plainly made up of a large number of smaller granules.

This fusion of the threads and of the chromomeres occurs very early in the period of synapsis; but after the fusion the synaptic condition persists, certainly for days, perhaps for a week or more. Toward the end of this period, the aggregation of the spirem becomes gradually looser, and there follows a stage in which the thread is very evenly distributed throughout the nuclear cavity, and is in côntact with the nuclear membrane at very many points.

While the spirem is in this distributed condition, it becomes longitudinally split, the splitting of the thread being preceded by a fission of each chromomere. The halves of the split thread are much twisted about each other, and, except for the manner of its distribution within the nucleus, the appearance of the spirem at this stage is in all respects strikingly similar to its appearance immediately before the original fusion.

There now ensues another change in arrangement; the thread is drawn inward from many of its points of contact with the nuclear membrane, and the greater proportion of its mass becomes aggregated in the central portion of the nuclear cavity, giving rise to a figure

which recalls that of synapsis. This later stage of aggregation was figured and described by Miss Sargant ('96, '97) as a "second synapsis;" and it is the stage described as synapsis by Ernst ('02), who apparently did not see the synapsis stage, properly so-called.

Those portions of the split thread which lie in the peripheral region of the nucleus at this stage consist of loops which originate in, and return to, the central mass. The number of such loops is twelve. Transverse segmentation now occurs by the breaking apart of each loop in its peripheral region; each chromosome when first formed has its ends, therefore, at the periphery, and its median portion involved in the apparently tangled mass in the center of the nucleus. This is the general rule; but sometimes a chromosome lies comparatively free from its fellows, so that it can be followed throughout its length.

An arrangement of the spirem into loops just before segmentation has been found by Schaffner ('97) in the macrospore mother-cell of *Lilium philadelphicum*, and by Farmer and Moore ('03) in the heterotypic divisions of both animals and plants; but, according to their descriptions, segmentation occurs somewhere in the central region of the nucleus, so that the peripheral portion of each loop becomes the central part of one of the newly-formed chromosomes. The looping, as they conceive it, is preparatory to the bending of each chromosome into two closely appressed arms, which are destined to be separated in the metaphases by a transverse fission. My figures agree with those of both Schaffner and Farmer and Moore as to the formation of the loops; but it is certain that in *Lilium canadense* a loop does not represent that part of the spirem which is destined to form a chromosome; but that, on the contrary, the peripheral portion of the loop marks the region in which the separation between two adjacent chromosomes is to occur. The looping, therefore, has nothing to do with a folding or bending of the chromosome, which, in fact, according to my observations, never occurs.

It will be seen that each chromosome consists, from the time of its formation, of two portions, the products of a longitudinal splitting, which are twisted about each other. This double nature persists throughout the period of shortening of the chromosomes, down to the time of separation of the daughter chromosomes in the metaphases; the separation in the heterotypic division, therefore, is along the line of the longitudinal fission which the spirem underwent before its segmentation.

My observations as to the history of the chromosomes after seg-

mentation, and as to the method of their separation, are in harmony with the more recent results of Strasburger ('00) and Mottier ('03). When the equatorial plate is formed, the majority of the chromosomes are attached at or near one end to the spindle; but a few are attached at the middle or at some point between the middle and one end. In the commoner case, that of the attachment at one end, the daughter chromosomes separate from each other as straight rods; but just before the completion of the separation, each daughter chromosome splits longitudinally, the halves diverging at their equatorial ends and remaining in contact by the ends directed toward the pole, giving a V-shape to the daughter chromosome. It is often evident that this second longitudinal split extends the full length of the daughter chromosome, the granddaughter chromosomes so produced remaining in contact, however, at their polar ends.

In the separation of the halves of a chromosome whose attachment is at or near the middle, each daughter chromosome becomes bent at the point of attachment and assumes, during the separation, a U or V-shape. When, therefore, the second longitudinal split occurs, such a daughter chromosome is divided into two V's, which remain in contact at their angles. This variation in the appearance of the daughter chromosomes, due to the method of their attachment to the spindle, has been responsible for much of the confusion that has existed regarding the nature of the heterotypic division in plants.

After the gathering of the daughter chromosomes at each pole of the spindle, they become curved, bent, and crowded together into a dense mass, about which a nuclear membrane is formed. The curving is such that a free end of one granddaughter chromosome (turned toward the equatorial plane of the spindle) comes into contact with a corresponding end of another chromosome. Whether these ends become fused, forming a continuous spirem, I have been unable to determine. At any rate, very early in the prophases of the homoeotypic division, some time before the disappearance of the nuclear membrane, the thread loosens and spreads apart to some extent, and it is then seen to be composed of segments, which have the shape of V's with curved arms, and which occupy exactly the same position as did the daughter chromosomes in the anaphases of the preceding division. There can be no doubt, I think, that the V-shaped chromosomes of the homoeotypic division are identical with the daughter chromosomes of the heterotypic division.

These V shaped chromosomes become arranged in the equatorial

plate with their angles turned toward the interior of the spindle, and it is often evident at this stage that the arms of a V are not fused, but are simply in contact at the angle. Sometimes more than twelve V's are to be observed. If, as I suspect, this fact is due to the persistence of the form of the occasional V-shaped granddaughter chromosomes of the previous division, we should expect to find now and then a V-shaped daughter chromosome in the metaphases of the homoeotypic division; but I have not as yet observed such figures. The separation in the metaphases is usually, at least, at the angle of the V, resulting in rod-shaped daughter chromosomes which are identical with the granddaughter chromosomes of the heterotypic division.

If my description be correct of the origin of the single spirem by a fusion of two originally separate threads, an explanation is offered for the sudden appearance in the heterotypic division of the reduced number of chromosomes. Each nucleus of the plant or animal, in preparing for division, forms a spirem, composed of substances derived in equal proportions from the male and female parents. There is no fusion of these hereditary substances thoughout the life history of the individual, until the initiation of nuclear division in the spore mother-cells in the case of plants, or in the primary spermatocyte or the primary oocyte in the case of animals. In the prophases of this division, the two portions of the spirem, each derived ultimately from one of the parent germ cells, become applied to each other and fuse into a single thread, so effecting the mixture or interaction of hereditary qualities which was provided for by the fusion of the sex cells at the beginning of the life-cycle.

As I have said, the chromomeres fuse in pairs; but just what this fusion involves as regards the smaller units which make up the chromomeres is a problem which at present perhaps cannot be solved by direct observation. If we adopt the hypothesis, frequently advanced, that the transmission of hereditary qualities is a function of the chromomeres, or of their component units, a very interesting parallel may be traced between the observed facts above described and the results of recent experimental studies of hybridization. A discussion of this and of related questions will be deferred until the appearance of the complete paper.

It is interesting to note that De Vries ('03) has recently concluded, upon hypothetical grounds, that before their separation in the heterotypic division the chromosomes lie side by side in pairs, each pair consisting of a paternal and a maternal segment; and that, in this condition

of intimate contact, a mutual interaction, or even an interchange of hereditary units occurs. The pairing of the parental chromosomes postulated by De Vries may be conceived to be effected by the method described by Farmer and Moore ('03), who hold that each apparently double chromosome is formed by a transverse bending of one of the original segments of the spirem. If it be maintained that one of the segments which thus folds upon itself represents a paternal and a maternal chromosome attached end to end, it would seem to follow that in the spirem of a somatic mitosis the parental elements are arranged in regular alternation. If the views expressed in the present paper be correct, it is more probable that the chromosomes derived from the male parent are attached end to end to form a thread, and that those from the female parent are arranged into a similar thread; and that in a somatic mitosis these two threads in turn unite by their ends to form a continuous spirem.

The peculiarities of the heterotypic division described by Rosenberg ('04) in a hybrid Drosera, in which ten single and ten double chromosomes appear, may be accounted for by supposing a fusion of the two parental portions of the spirem in the early prophases. The portion derived from one parent, containing only ten segments, would extend only half the length of the other portion, containing twenty segments; the thread on segmenting would therefore give rise to ten segments of double thickness and ten of single thickness, the latter perhaps incapable, in this division at least, of longitudinal splitting. —CHARLES E. ALLEN, *University of Wisconsin, Madison, Wis.*

NOTE.—Since the above was written, there has appeared a further account by Rosenberg ('04 a) of the post-synaptic processes in Drosera. He is convinced that there is a fusion of the chromosomes side by side during the spirem stage. Strasburger ('04), on the other hand, has described in Thalictrum a quite different method of conjugation of the paternal and maternal chromatin in entire independence of the linin. A. and K. E. Schreiner ('04) have found a fusion and subsequent splitting of the spirem, substantially identical with the processes I have described, in the spermatogenesis of Myxine and Spinax; and similar results had been previously announced by von Winiwarter ('00) and Schoenfeld ('01) with respect to mammalian oogenesis and spermatogenesis.

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